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STUDIES ON EXPERIMENTAL SCURVY IN GUINEA-PIGS *

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The earlier literature relating to scurvy, both clinical and experimental, has been so recently and thoroughly reviewed by Holst and Fröhlich¹ and Hart and Lessing² that no detailed discussion of it is necessary here.

In 1907 Holst and Fröhlich were able by feeding guinea-pigs on bread, oats, and unpeeled grains to produce a condition which they stated to be in all essentials wholly identical with that found in human scurvy. In their more recent work they produced this disease by feeding a variety of different foods excluding fresh vegetables, the changes being essentially the same as in the first series.

Hart and Lessing² produced experimental scurvy in monkeys by feeding them with condensed milk to which had been added cooked rice and dried pig nuts (Erdnüsse). The first symptom noted was bleeding of the gums with later on loosening of the molar teeth unattended by much swelling or ulceration of the gums. Hemorrhages into the skin were not observed, but hemorrhages over swollen knees, ankles, or wrists were often met with, the swelling usually being accompanied with much pain. Spontaneous fractures occurred if life was sufficiently prolonged. The joints were normal.

These authors agree with Fraenkel that hemorrhage is invariably present and that it is not dependent on the existence of fractures but occurs much earlier, before there is any clinical manifestation of the disease. "Die Farbung des Blutergusses verbunden mit deutlicher Schnittenbildung weist darauf hin dass die Blutung subweis refolgt." In agreement with Schmorl and Fraenkel the authors believe that the changed bone marrow, "Gerüstmark," is not dependent on hemorrhage, as held by Looser and Jacobsthal. They think that the primary "Krankheitsnoxe," the nature of which is unknown, exerts a constant influence not only on the origin but on the further development of "Gerüstmark" and thus produces the varying picture. They note a great poverty of blood vessels in the fibrous marrow, to which they attribute the disturbances in endochondral ossification. The general disturbances in bone-production are not caused, they think, by the marrow changes, but are due to a general affection of the whole organism, as Schmorl and others have stated. The changes they observed in the periosteum were small hemorrhages without thickening, and larger hemorrhages which resulted in a thickening of the periosteum. Holst and Fröhlich found in guinea-pigs atrophy and fatty degenera-

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¹ Jour. Hyg., 1907, 7, p. 619. Ztschr. f. Hyg. u. Infektionskrankh., 1912, 72, p. 1.

² Der Skorbut der kleinen Kinder, 1913.

tion of the muscle fibers and also disintegration into irregular hyaline droplets and irregular staining properties. Hart and Lessing found hydropic degeneration but no fatty degeneration, and in some fibers a deposition of fine granules of calcium. From their experiments on monkeys Hart and Lessing conclude that the skeletal changes produced are identical with those of scurvy but not with those of rickets.

Pappenheimer³ working with thymectomized rats observed extensive changes in the bones and teeth of a number of animals, not alone of the thymectomized rats, but of controls and stock as well, and from a study of the condition he believes "that the alterations of the skeletal system are not due to the lack of thymus secretion but are a result of spontaneous disease first described by Morpurgo." He found changes in the zone of proliferation of the long bones and ribs, an increase in width of the cartilage and an irregular proliferation of cartilage cells, the presence of osteoid tissue, density of the spongiosa, with narrowing of the marrow cavities and thickening of the compacta; also marked changes in the teeth, defective and irregular calcification of the dentin, and in some cases hyperemia and necrosis of the pulp. In a later publication⁴ he describes the production of rickets in rats by the injection of a suspension of bone marrow of a rachitic animal.

The importance of circulatory disturbances in accounting for the alterations found is generally recognized. Morpurgo⁵ states that "Ueber die Pathogenese der Krankheit werde ich nur hervorheben, dass die circulatorische Verhältnisse von Grosses Deutung sind." More recently from a clinical study of many cases of human scurvy Hess⁶ arrives at the conclusion that hemorrhages in scurvy "are attributable to a disturbance of the vessel walls rather than to a defect in the normal coagulation of the blood." He has not, however, informed us as to the nature of these changes.

Experimental scurvy has been most often produced by diets of grains. The feeding of milk from one animal species to others has been employed less frequently, with results which differ considerably. Keller⁷ and Lane-Clayton⁸ could observe no difference between mice and rats fed on raw and those fed on cooked cow's milk; Peiper and Eichloff⁹ found that dogs fed on raw and sterilized milk developed a fragility of the bone and small hemorrhages around the epiphyseal borders. Rodet,¹⁰ Bruning,¹¹ and Moro¹² do not describe such changes. Rabbits, according to Moro, cannot live on cow's or woman's milk. Young pigs, as noted from Bruning's observations, appear to be better nourished by raw than by cooked cow's milk.

Bolle¹³ was apparently the first to call attention to a condition in guinea-pigs, having many features similar to infantile scurvy, which was produced by feeding milk. Bartenstein,¹⁴ feeding cooked and raw cow's milk to guinea-pigs, observed similar changes. Fröhlich,¹ repeating these experiments in a more

³ Jour. Exper. Med., 1914, 19, p. 319.

⁴ Ibid., 20, p. 477.

⁵ Verhandl. d. deutsch. Gesellsch., 1900, 3, p. 40.

⁶ Proc. Soc. Exper. Biol. and Med., 1913-14, 2, p. 130. Am. Jour. Dis. Child., 1914, 8, p. 385. Jour. Am. Med. Assn., 1915, 65, p. 1003.

⁷ Ztschr. f. diätet. u. physik. Therap., 1904, 7, p. 90.

⁸ Jour. Hyg., 1909, 9, p. 233.

⁹ Quoted from Funk, Die Vitamine, 1914, p. 87.

¹⁰ Compt. rend. Soc. de biol., 1896, 48, p. 55.

¹¹ Wien. klin. Rundschau., 1904, 27, p. 481.

¹² München. med. Wehnschr., 1907, 54, p. 2223.

¹³ Ztschr. f. diätet. u. physik. Therap., 1902-03, 6, p. 354.

¹⁴ Jahrb. f. Kinderh., 1905, 61, p. 22.

intense study, concluded that raw and cooked milk produced similar conditions, but that cooked milk produced severer lesions. Holst and Fröhlich contended that the lesions produced by feeding grain are different from those caused by feeding milk. Funk¹⁵ prevented the onset of scurvy and loss of weight by feeding guinea-pigs 50 c.c. of uncooked milk daily in addition to the oats diet. After elimination of the caseinogen and other proteins, the milk still retained its antiscorbutic properties. Attempts to isolate this antiscorbutic substance were unsuccessful.

The occurrence some time ago of a peculiar affection in a number of guinea-pigs in the laboratory of St. Luke's Hospital, previously described by Davis and Moore,¹⁶ led to the present studies concerning the effect of milk diets on guinea-pigs. Nine of 26 guinea-pigs in the animal room at that time appeared partially paralyzed, dragging their legs and moving about the cages with difficulty. The muscles of the legs and the tissues, especially around the wrist and knee joints, were swollen and extremely tender on pressure. The animals showed some emaciation. An epidemic disease of some kind was suspected.

On investigation we found that the diet of the affected guinea-pigs, in addition to oats, green vegetables, hay, and water, consisted largely of milk from a cow having experimental streptococcal mastitis. We at once became suspicious of the milk diet, as there had been no such disease before its use. Experiments were designed to ascertain what factors in the diet were responsible. Groups of animals were fed on various combinations of food chiefly in relation to milk or milk constituents to test both the production of the disease and its prevention. These groups will be considered separately.

GUINEA-PIGS FED ON PASTEURIZED MARKET MILK

This milk was purchased directly from the dealers and fed to the guinea-pigs within 24 hours after delivery. The milk had been pasteurized for from 25 to 30 minutes at 104 F. The bacterial count at the time of delivery averaged 20,000 to the cubic centimeter. The bedding in the cages was timothy hay. Thirty-two animals were fed on this milk for 18 or more days. Five remained well. The usual history of the others was as follows:

The guinea-pigs for the first 2 or 3 days did not apparently care for milk, but by the 4th day and from then on they drank it with great avidity. They gained in weight and appeared to be in the best of physical condition. At different dates varying from 10 to 29 days the wrist joints became painful to pressure and palpable swelling followed in the course of from 24 to 48 hours. Occa-

¹⁵ *Biochem. Jour.*, 1913, 7, p. 81. Also *Die Vitamine*, 1914.

¹⁶ *Tr. Chicago Path. Soc.*, 1914, 9, p. 185.

sionally swelling was found with the first signs of tenderness. The joints continued to enlarge until in some instances they were from two to three times their normal size. After 2 or 3 days, the knee joints underwent similar changes while occasionally the ankle, elbow, and shoulder joints became affected in the order named. While the swelling at the wrist joint was at first observed to be more closely confined to the ends of the ulna and radius, that at the knee joint was about the heads of the tibia and fibula. Altho the swelling occasionally spread to encompass the entire joint and even into the tissues of

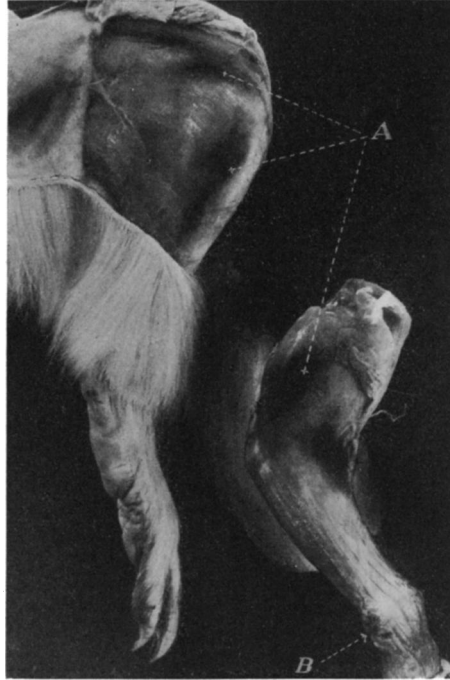


Fig. 1. Intramuscular (A) and subcutaneous hemorrhage with swollen wrist joint (B) in Guinea-pig 186 fed for 41 days on raw certified milk.

the leg and calf, it was most persistent around the tibia and it was at this location that subsequent changes were most frequent. The calves of the hind legs were frequently swollen and the skin over the affected muscles sometimes turned blue (seen most in white pigs), and underwent the changes commonly observed following bruises. At first the swollen parts sometimes were soft, sometimes firm like a brawny induration. In animals with a sudden and large swelling, the tissues were softer; where the swelling increased more slowly, a firm hard mass was the result. An enlargement of the costochondral junctions resembling a rachitic rosary often was palpable a week after the initial symptoms, and it developed with little noticeable swelling of the surrounding tissues.

At the commencement of symptoms the animals usually appeared to be depressed, became more or less emaciated, and, if the disease progressed, sometimes died. Only 4 guinea-pigs of this series, however, died spontaneously

from the scorbutic condition *per se*; these died on the 8th, 29th, 34th, and 35th days after the onset of the disease, and on the 26th, 44th, 55th, and 57th days after commencing the milk diet. Two others died of pneumonia, one on the 66th, the other on the 90th day. The latter had swollen joints on the 18th day, the former had no palpable joints, but old hemorrhages were found about the knee joints at autopsy. Twelve animals were killed from the 18th to the 31st day. Seven of these had lesions around the wrist joints. Fourteen after feeding on milk for a long time were placed on a general diet. Five had no palpable lesions altho fed on milk for from 30 to 146 days. A possible explanation of this will be discussed under the changes in weight of the animals.

The average time for the appearance of swellings or hemorrhages around the joints was 19 days. This factor was variable, however, depending to some extent on the weight and the age of the animal, the heavier and older pigs as a general rule not having lesions as soon as the lighter and younger ones. The earliest lesions were observed in 10 days. The longest period for their development was 29 days. In the majority of animals the first joint enlargement was found between the 18th and 22nd days.

The gross alterations varied considerably, depending on the age and the period of feeding. The most constant alteration found at autopsy was hemorrhage. Hemorrhages were most frequent around wrist joints and in the muscles of the hind legs (Fig. 1). They might be few and small. Occasionally the only lesion was a small hemorrhage into the periarticular tissue of the wrists or into the capsule of the anterior tibial tendon of the knee joint. Hemorrhage with accompanying edema, or edema only, was apparently the cause of the primary swelling of the wrist and knee joints. Extensive hemorrhages into the thigh muscles extending around the hip joints were more rarely observed; subcutaneously they were found commonly in the hind limbs, less frequently in the jaw muscles, almost constantly in the bone marrow around the ends of the long bones, and in a few instances within the joint cavity.

At the wrist joints (Fig. 1), the ends of the radius and ulna were enlarged, yellowish white in color, with the consistency of cartilage. At the knee joint, the upper ends of the tibia and fibula had a similar enlargement; more rarely the lower ends of the tibia, fibula, and femur were enlarged. The bones became fragile, often being fractured in manipulation. Fracture occurred generally in the lower third of the tibia and fibula. More commonly we found fractures at the epiphyseal junctions of the radius, and ulna at the wrists, and of the tibia at the knee. Infractures were sometimes found with the periosteum holding the bone in place. The fractures produced various deformities, which sometimes entirely disappeared after healing.

Swelling of the costochondral junctions with or without hemorrhages was occasionally observed, producing a "rachitic rosary" comparable clinically to that of rachitis in human beings (Fig. 2).

In the region of the hemorrhages there occurred an edema, usually limited in extent. Frequently there was an edema around the inguinal and axillary lymph glands with enlargement of the glands, especially the inguinal group. About 75% of the animals had a definite swelling of these glands with occasional hemorrhage into the gland substance.

The spleen was frequently enlarged. In general the viscera possessed no important gross lesions. We found hemorrhage and ulceration of the stomach in one animal and hemorrhages into the adrenals once. Fragility of the lower maxilla with loosening of the molars, which is often observed when feeding guinea-pigs on various grain diets, was not found. The only changes in the gums in this group were the appearance of a submucous hemorrhage at the

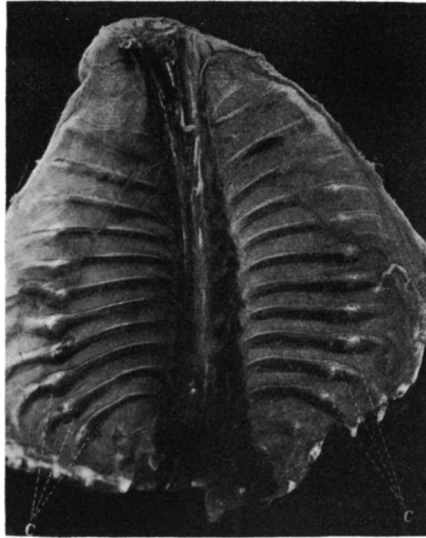


Fig. 2. Enlargement of the costochondral junctions (C) in Guinea-pig 186 fed for 41 days on raw certified milk.

base of the lower incisors of 1 pig, and hyperemia of 3 others. The glistening white color of the lower incisors was occasionally replaced by a dull yellow color extending two thirds of the distance from the base to the end. Petechiae into the follicles of the vibrissae of the lips and muzzle were not found grossly or microscopically.

We have previously mentioned that 5 animals, altho fed for from 30 to 146 days on milk, showed no palpable lesions. The animal fed for 146 days weighed 235 gm. at the beginning of the experiment. The other four were not weighed, but were large guinea-pigs approximately over 300 gm. The average weight of those having lesions was 120 gm., the lowest being 80 and the largest 480.

The weight of the guinea-pigs is important as an index of the age. The age of the guinea-pigs when first fed varied between 2 and 5 weeks, and the lesions occurred most frequently in young animals in which the process of bone and tissue building was at its maximum; in but one instance were symptoms of the disease observed in older animals—a guinea-pig weighing 480 gm. which had swollen wrists on the 17th day. That such a diet occasionally produced a somewhat similar, tho less marked, effect on the older animals was again demonstrated in small old healing hemorrhages around the knee in a guinea-pig which had been fed for 66 days on pasteurized milk with no symptoms of the disease during life. More recent experiments have shown that large guinea-pigs are almost as susceptible as small ones.

The time of appearance of the lesions was variable, depending on the age and weight of the animal. In one group averaging 138 gm. in weight, the first lesions were observed on the 26th to the 29th days; in a second group averaging 130 gm., on the 18th day; and in a third group averaging 120 gm., on the 10th to the 13th days. Of the 4 animals which died, 3 weighed 100 gm. and 1 weighed 135 gm. at the beginning of the experiment.

The following specific case is typical of this group of animals:

Guinea-pig 58.—Commenced feeding on pasteurized market milk March 16. Weight 100 gm. On the 15th day both wrists slightly swollen and tender. Weight 205 gm. The wrists rapidly increased in size, and on the next day the knee joints, especially around the heads of the tibia, were noticeably enlarged. The animal died on the 24th day. Weight 150 gm. Necropsy: Periarticular tissues around the wrist joints swollen and filled with blood. Some hemorrhage into the muscles near the joint. Subcutaneous and intramuscular hemorrhage about the knee joints, which were almost twice normal size. Enlargement of the costochondral junctions with no hemorrhage. Inguinal lymph glands swollen and hemorrhagic. Lungs congested, with a small hemorrhage in one lobe. Other organs normal.

GUINEA-PIGS FED ON RAW MILK

It was found by Funk, Fröhlich, and others, that raw milk contains more of the vitamins, or antiscorbutic substances, than are contained in pasteurized and cooked milks. Fröhlich states that milk pasteurized at 70 C. for 30 minutes preserves a large but uncertain portion of its antiscorbutic properties, while milk heated for 10 minutes at 98 C. completely loses this property. We therefore fed small guinea-pigs on raw certified milk.

Nine guinea-pigs fed on this milk developed scurvy by the 22nd day. One group of 3, averaging 87 gm. in weight, had lesions on the 10th to the 12th days. Two of these died, one on the 17th, and one on the 19th day. The second group of 4 weighed 90, 125, 140, and 155 gm. respectively. The smallest guinea-pig had lesions on the 10th day, the others on the 11th day. The guinea-pig weighing 125 gm. died on the 23rd day, the largest on the 48th day. The other two were killed. A third set of 2 weighing 160 gm. and 150 gm. respectively, had lesions on the 22nd day. One died on the 28th day.

The lesions were similar to those of the preceding group. All the animals had enlarged wrists and costochondral junctions. Two had hyperemia and one had petechial hemorrhages at the base of the lower incisors. Fragility of the maxillae and loosening of the molars were not observed.

It is apparent from this experiment that a diet of raw milk is not sufficient to prevent experimental scurvy in young guinea-pigs. The protocol of one of the animals follows:

Guinea-pig 188.—Commenced feeding on certified milk February 3. Weight 155 gm. After 11 days the wrists were enlarged and painful. Weight 180 gm. The wrists increased in size and the forelegs from elbow to wrists became swollen. At the same time the knees, especially around the tibia, were enlarged and painful. By the 28th day the enlargement of the costochondral junctions was marked and easily palpable. At this time the weight was 160 gm. The swellings of the joints increased in size up to the 31st day, gradually becoming more firm. On the 37th day 2 small hemorrhages were observed at the base of the lower incisors. These disappeared in 2 days. The enlarged joints slowly decreased in size, the swollen portions taking on the appearance of exostoses. Death on the 48th day. Weight 170 gm. Necropsy: Hard appar-

ently bony swellings around the ends of the radius and ulna of both wrists, and at both ends of the tibia and fibula of both legs, more marked at the knee than at the ankles. Yellowish enlargements of the costochondral junctions, with no hemorrhages. Old hemorrhages in the tissues and muscles around the knees. Enlarged inguinal glands. No other changes found.

GUINEA-PIGS FED ON BOILED MILK

Eight animals were fed for a sufficient time for the development of scurvy lesions on milk boiled for 10 minutes. Milk subjected to this process has its entire antiscorbutic property destroyed, according to Fröhlich.

Three of the animals were weighed during the experiment. At the start of the experiment their weights were 115, 115, and 190 gm. respectively. The first lesions were observed on the 23rd, 24th, and 24th days respectively.

One of the 8 guinea-pigs (Guinea-pig 45) died from the disease, two were killed accidentally, and one was chloroformed. The remainder were placed on a vegetable diet after 2 months. Two of the animals did not develop palpable lesions altho fed for 66 days on boiled milk. These were large ones in the same group as those fed on pasteurized milk for 2 similar periods with no gross lesions developing.

The shortest period elapsing before the appearance of joint lesions was 13 days, the longest 24 days; the average for the 6 guinea-pigs was 19 days. It is to be noted that the animal weighing 190 gm. showed symptoms of the disease on the same day as the one weighing 115 gm.

An instructive protocol follows:

Guinea-pig 45.—Commenced feeding on milk boiled for 10 minutes January 9. Both wrists swollen on the 13th day. These enlarged rapidly and became firm. Death occurred on the 39th day. Necropsy: Lungs congested. Eight to 10 hemorrhages measuring about 1 mm. in diameter on the posterior wall of the stomach. Mesenteric lymph glands swollen and hemorrhagic. Axillary and inguinal glands enlarged. Periarticular tissues of both wrists and of the right elbow contained hemorrhages. The lower ends of the tibia and fibula markedly swollen and hard. Costochondral junctions slightly enlarged. Other tissues and organs normal.

GUINEA-PIGS FED ON MILK AND STREPTOCOCCUS BROTH

Since the guinea-pigs in which we first observed the symptoms of scurvy had fed on milk from a cow with experimental streptococcal mastitis,¹⁷ it was thought wise to ascertain whether streptococci added to milk would produce more rapid or severer lesions.

Seven guinea-pigs were therefore fed on pasteurized market milk to which for each guinea-pig, 20 c.c. of a 24-to-48-hour broth culture of a streptococcus were added daily. Three others were fed on milk boiled for 10 minutes to which a like portion of the streptococcus broth was added. Both *Streptococcus hemolyticus* and *Streptococcus viridans* were grown in the broth. These had

¹⁷ Jour. Infect. Dis., 1914, 15, p. 135.

been isolated from patients with tonsillitis, arthritis, endocarditis and various other conditions.

In the first group the earliest lesions appeared on the 12th day, the latest on the 22nd day, average 17 days. In the second group the first appearance was on the 13th day, the latest on the 19th day, average 16 days. The symptoms and lesions were similar in all respects to those previously described. Two of the animals had their throats swabbed daily with streptococcus broth, but we observed no difference in symptoms between these and those not swabbed. One animal died on the 26th day, 5 days after the appearance of the lesions. For an example of this group the reader is referred to the report on Guinea-pig 16 under the discussion of temperature and blood changes.

GUINEA-PIGS FED ON STREPTOCOCCUS BROTH

If a streptococcal infection is the sole cause of the disease, as is suggested by the experiments of Morpurgo⁵ and Koch,¹⁸ then animals fed exclusively on broth cultures of streptococci possibly should have earlier and severer lesions. Mixed cultures of hemolytic and green-growing streptococci were grown in broth for from 24 to 48 hours and this broth fed daily to the animals.

All of 8 animals died except one. The latter had a broth diet with hay for 21 days, after which it was placed on a diet of mixed vegetables in addition to the broth. Four of the animals died on the 8th and 9th days. Three died on the 19th, 29th, and 33rd days. Two animals lived for a sufficient length of time to have lesions—that is, 29 and 33 days—but in neither animal did we observe any hemorrhages around the joints usually affected or any swelling of the ends of the long bones. In all these animals we found at autopsy a subcutaneous edema with more or less clear fluid in the peritoneal cavity. In general the lymph glands were larger than normal. Congestion of the lungs with some pneumonia was a feature in each instance. The molars were not loosened, nor were there any hemorrhages in the muscles of the jaw. Macroscopical examination revealed no evidence of scurvy. There was, moreover, slight or no increase in weight.

Cultures of the intestinal tract of these animals contained streptococci of the varieties given in the diet. Cultures from the stomach of 2 fed on oats, and from the stomach and various portions of the large and small intestines of 2 fed on milk, and of normal pigs, contained no streptococci.

GUINEA-PIGS FED ON WATER AND HAY

Three guinea-pigs were fed on water and hay. Two were fed for 18 days with no consequent scorbutic lesions and then were placed on a vegetable diet. The third died on the 14th day. The only gross pathologic change was a moderate edema of the subcutaneous tissue with marked emaciation. No gross lesions of scurvy were observed.

In a similar experiment by Holst and Fröhlich¹ the animals had a marked universal subcutaneous edema with some ascites. A few small hemorrhages were found in the muscles and subcutaneous tissues but

¹⁸ Centralbl. f. Bakteriöl., R., 1913, 57, p. 250.

microscopically none of the tissue contained any indications of a scorbutic affection; the bone, however, had a pronounced starvation marrow.

GUINEA-PIGS FED ON CARROTS, CABBAGE, AND HAY

Seventeen guinea-pigs were fed on carrots, cabbage, and hay for from 33 days to 4 months. They developed normally, with no symptoms of scurvy. This is the usual diet fed to our guinea-pigs, and on it none has ever developed scurvy.

GUINEA-PIGS FED ON MILK, VEGETABLES, AND OATS

Five guinea-pigs were fed on milk, carrots, cabbage, lettuce, hay, and oats. All were on the diet a sufficient length of time to have lesions of scurvy.

Two weighing 155 and 165 gm. respectively had no gross lesions after 4 months on such a diet. One guinea-pig weighing 175 gm. had swollen wrists by the 21st day. These went on to exostoses, the animal having mild symptoms and continuing to gain in weight. Two others weighing 90 and 95 gm. had symptoms first appearing on the 41st day. Three others weighing 110, 141, and 160 gm. were fed on milk and carrots for 50 days, with no consequent pathologic changes. It is to be noted that the smallest animals had the lesions.

A protocol of one of the three animals affected follows:

Guinea-pig 94.—Commenced feeding on pasteurized milk, vegetables, and oats April 9. Weight 90 gm. On the 41st day both wrists were swollen and painful. Weight 330 gm. During the following 4 days the animal lost 40 gm. The swollen wrists became firmer, and in a week the enlarged ends of the radius and ulna could be distinguished. After 3 weeks these became apparently bony exostoses and persisted for 4 months. The animal regained its former weight by the 67th day, after which it gained at the normal rate, weighing 400 gm. at the end of 4 months.

It is highly probable that those animals having scurvy drank more milk than the ones not so affected. As these experiments were conducted on groups and not on single animals, we have as yet not determined this factor.

At this point it might be well to reconsider the first outbreak of scorbutic symptoms which occurred in the laboratory guinea-pigs and which led to the present studies. As previously stated, 26 guinea-pigs were in the animal room when we first discovered the condition; 9 of these had symptoms of the disease. Eleven small guinea-pigs including the 9 affected had been purchased about 25 days preceding the outbreak. The actual weights of these were not determined, but we judged that they weighed from 150 to 250 gm. Fifteen animals had

been in the room for several months. None of these was affected as far as a physical examination could determine.

The animals were kept in groups in separate cages. Some had been inoculated with questionable tuberculous fluids and other material. The majority, however, were normal. The diet consisted of oats, corn, green vegetables such as lettuce tops, cabbage, and carrot tops from the kitchen, and raw milk from a cow with experimental streptococcal mastitis. All the animals had the same diet. The milk at first contained enormous numbers of streptococci, but had been free from cocci for 10 days before the first animal appeared sick. The milk had been fed to the animals for over 6 weeks. The diet with the exception of the milk had been the ordinary food of the guinea-pigs for several years. As soon as we suspected the milk as the causative factor in the condition, it was removed from the regular dietary, and thereafter no symptoms of scurvy appeared among the guinea-pigs.

Taking under consideration the preceding experiments, we have a possible explanation for the outbreak. We have demonstrated that milk, whether cooked, pasteurized, or raw, will produce clinical scorbutic lesions in young guinea-pigs in periods varying from 13 to 28 days. Secondly, we have demonstrated that when milk is added to the ordinary vegetable diet of guinea-pigs (the latter diet never having produced scurvy), a certain number of the animals will have clinical evidence of scurvy. But the animals which are usually affected are the young growing guinea-pigs, weighing less than 200 gm. As a general observation it can be stated that the younger the animal, the more severe the lesions. The guinea-pigs which had the lesions were all small. None of the large animals were affected, altho they had been having milk for about 6 weeks. The sick animals had been having milk for about 25 days when the symptoms were observed. This is about the length of time after feeding that we found scurvy produced in the experiments. The pathology was identical with that found in the guinea-pigs in our experiments, as is illustrated by the following protocol:

Guinea-pig 3.—On May 24 walked with difficulty, hind legs appearing stiff. Knee joints swollen and tender. The knee joints increased in size during the following 5 days, and the right leg could not be straightened.

May 31.—Right wrist joint swollen. Right knee joint aspirated, small amount of blood being obtained. Temperature 101.

May 2 and 3.—Right shoulder joint swollen. All the costochondral junctions enlarged. Right wrist almost normal in size. Both knee joints smaller than before and firm. Chloroformed. Bled from heart and the blood injected into Guinea-pig 8. Culture of heart blood sterile.

Necropsy: Mesenteric, inguinal, and axillary lymph glands swollen. Tissues around right knee joint swollen, containing many hemorrhages. Lymph glands in the inguinal space greatly enlarged. Left knee joint similar in appearance to the right except that there were fewer hemorrhages and less swelling. No pus in the joint cavities; the articular surfaces normal in appearance. Costochondral junctions swollen but no accompanying hemorrhages. Right wrist slightly swollen and much congested. Abdominal organs normal. Cultures from the knee joints sterile.

Cultures from the heart blood from several of these animals were sterile. Of cultures from the joints, one yielded a hemolytic streptococcus, but as the animal in this case was on a streptococcus milk diet we could not rule out a contamination. The others were sterile. Four times blood was aspirated from the heart of a sick guinea-pig and injected into the peritoneal cavities of other guinea-pigs in quantities varying from 1.5 to 5 c.c., but in only one instance did an inoculated animal have similar lesions, and this occurred before we eliminated milk from the general diet. None of the three animals inoculated and kept on an antiscorbutic diet had any symptom of scurvy.

After ascertaining that milk either had something in it which caused scurvy or lacked some essential which prevented the disease, we undertook a series of experiments to learn if possible with which constituent of the milk this property was associated.

GUINEA-PIGS FED ON SKIM MILK

If the fat content of milk was responsible, either raising or lowering this content might prevent or increase the symptoms. According to Funk,¹⁹ milk after removal of its fat by centrifugation loses about 50% of its vitamins and allantoin.

Six guinea-pigs were fed on skim milk which had a fat content of 0.1%. One died of pneumonia in 8 days. Two weighing respectively 120 and 110 gm. at the beginning of the experiment did not have lesions after being on the skim-milk diet for 70 days. The others, weighing 90, 100, and 110 gm., had lesions on the 31st, 18th, and 18th days respectively. One died on the 21st day with the following lesions: edema of axillary regions with enlarged congested axillary lymph glands; swelling and congestion of inguinal glands; swellings around both wrists with periarticular hemorrhages; hemorrhages in the muscles of the neck and under the jaw; swelling of costochondral junctions, and petechial hemorrhages in the stomach wall.

GUINEA-PIGS FED ON CREAM

Five guinea-pigs weighing from 120 to 160 gm. were fed on cream with a fat content of from 26 to 28%. All lost weight rapidly and died, the periods varying from 3 to 20 days, with no macroscopic

¹⁹ Biochem. Jour., 1913, 7, p. 211.

lesions of scurvy. In every case the large intestine was distended with light mustard-colored semisolid feces. Apparently the digestive apparatus of the guinea-pig could not properly care for such a large amount of fat. The lipolytic enzymes seemed to be deficient. One would expect this in a herbivorous animal which has to digest only small amounts of vegetable fats.

GUINEA-PIGS FED ON MILK AND OLIVE OIL

After we had learned that cream was unsatisfactory, a vegetable fat, olive oil, was added to skim milk. The percentage of oil in this mixture was approximately the same as that of fat in cream. The six animals weighing from 100 to 150 gm. lost rapidly in weight and 5 had died by the 5th day, 1 living 11 days. The pathologic findings were similar to those of the guinea-pigs on cream diet. It was conclusively demonstrated that small guinea-pigs would not live on a diet with such high vegetable-fat content.

Olive-Oil Injections.—Since the animals did so poorly on cream and olive-oil diets, we considered the possibility of the prevention of the disease by injections of olive oil. Three guinea-pigs averaging from 120 to 140 gm. in weight were fed with pasteurized milk, and 0.5 c.c. of sterile olive oil was injected subcutaneously each day. All had symptoms of scurvy and swollen wrists between the 15th and 17th days.

GUINEA-PIGS FED ON LACTOSE WATER AND HAY

Funk found that in the case of animals on a scurvy-producing diet with antiscorbutic treatment an increase in the carbohydrates necessitated a proportional increase in the antiscorbutics to prevent the development of the disease. In other words, when he increased the carbohydrates, he produced a greater susceptibility in the animals toward scurvy. In order to learn whether the sugar in milk alone could produce scurvy 3 guinea-pigs were fed on 15% lactose water and hay. These were small animals weighing 110, 140, and 150 gm. Two died on the 15th and 16th days with congested lungs; the third lived 31 days. These had no pathologic lesions of scurvy but had the lesions of a starvation diet. They gained very little in weight.

GUINEA-PIGS FED ON LIME WATER

It has been held by several authors that the rarefaction of bone is due to a lack of lime salts.

Wright²⁰ believes this is produced by an acid intoxication and states that he found a diminution of alkali in the blood in 7 cases of scurvy. Stoezner,²¹ when feeding rabbits carbonate of lime, found a pronounced apposition of newly formed bones, while when feeding with oats alone he observed rarefaction and defective apposition. Holst and Fröhlich¹ fed 28 guinea-pigs with carbonate of lime in addition to bread, oats, and other grains with neither prevention of scurvy lesions nor diminution in the bone changes.

We fed 3 guinea-pigs with lime water and hay, the water being saturated with calcium carbonate. Two animals died on the 18th day with no evidence of scurvy, but with edema of the subcutaneous tissue and with some fluid in the peritoneal cavity. The third was placed on a vegetable diet on the 14th day. Two other guinea-pigs were fed daily with 1 gm. of calcium lactate in 150 c.c. of milk. The wrist and knee joints were enlarged on the 37th and 41st days, somewhat later than the appearance of lesions in the case of the milk diet alone. The animals both had severe lesions with swelling of both wrists and knees and fractures of a tibia in each instance.

The data in this experiment, altho few, indicate that calcium lactate will not prevent scurvy.

CASEIN AND HAY

Casein (10 gm.) in 150 c.c. of water was fed to 3 guinea-pigs. One died of pneumonia in 8 days. The others after 24 days, during which they gained no appreciable amount, and had swelling of the wrists; one died on the 27th day, the other on the 31st. Autopsy disclosed the following lesions:

Guinea-pig 101.—Fed on casein, water, and hay. Died on 27th day. Small hemorrhages around wrist joints and over the heads of both tibia. Slight but distinct enlargement of costochondral junction. Hemorrhages about 1 cm. in diameter into masseter muscles of both jaws and at base of lower incisors, and in large intestine. Lower ends of long bones at the wrist slightly swollen. The last two molars on either side were loose. Lungs congested. Spleen enlarged.

Casein, as is seen from the observations on these guinea-pigs, will produce a severer type of scorbutic lesion than we find occurring with any of the milk diets, as is evidenced by the loosening of the molars with hemorrhage into the jaw muscles. Of the different constituents of milk that were tested, casein was the only one which produced scurvy. Further studies along this line are now in progress.

CONDENSED MILK

Two guinea-pigs fed on condensed milk mixed with an equal amount of water had swollen wrists on the 15th and 16th days. Both died on the 21st day. The protocol of one follows:

²⁰ Lancet, 1900, 2, p. 565.

²¹ Virchows Arch. f. path. Anat., 1897, 147, p. 430.

Guinea-pig 120.—Commenced feeding on condensed milk diluted with an equal portion of water April 27. Weight 130 gm. Wrist swollen on 16th day. Weight 140 gm. Found dead on 21st day. • Weight 120 gm. Necropsy: Hemorrhages into muscles around knee and hip joints. Ends of tubular bones of the wrists slightly enlarged. Marked hyperemia of bony portion of costochondral junctions with some increase in size of the junctions and a widened line of ossification. Last molar teeth loose but no hemorrhages in gums. Spleen weighed 320 mg. in comparison with 130 mg for the spleen of a normal guinea-pig of the same weight. Adrenals weighed 100 mg. in comparison with 20 mg. for the adrenals of a normal guinea-pig. Other organs normal.

The adrenals and spleen of Guinea-pig 121 weighed 210 mg. and 350 mg. respectively. In both instances there was great enlargement of these organs. The lesions were severer than those found occurring in the case of other milk diets, if looseness of the molar teeth is taken as a criterion of severity.

THYROID EXTRACT, MILK, AND HAY

It was thought possible that the scorbutic condition might be due to some change such as a deficiency in the internal secretions of the body. Four guinea-pigs were therefore fed on milk and every 2nd day given 2 grains of desiccated thyroids. Three died on the 5th, 9th and 11th days respectively with no gross lesions of scurvy; the fourth had swollen wrists on the 11th day and died on the 15th day revealing typical scurvy lesions at necropsy.

GUINEA-PIGS FED ON OATS AND HAY, BREAD AND HAY, AND BRAN AND HAY

The studies of numerous authors have proved that certain grains when exclusively fed to guinea-pigs, will produce very severe lesions.

Holst and Fröhlich placed animals weighing between 300 and 600 gm. on diets of oats, rye, wheat, barley, oats, and groats. Death occurred in 30 days on an average with the symptoms of scurvy previously described but with severer lesions, looseness of the molar teeth occurring in all cases and hyperemia with swollen gums in 18%. Some of the guinea-pigs had hemorrhages beneath the mucous membrane; none had ulcerations of the gums.

As controls for the guinea-pigs fed on milk we placed 11 guinea-pigs on oats, hay, and water. They averaged 140 gm. in weight. The first symptom of scurvy appeared as swelling of the wrists within, on an average, 14 days. In one group of 3 the average was 11 days, in a second group of 3, the average was 19 days, a variation similar to that found in the milk-diet tests. Death occurred on an average within 32 days. In every instance looseness of the molar teeth was apparent, accompanied in several by hemorrhages into the gums or into the muscles of the lower jaw. The extent of the lesions is well portrayed in the following protocol:

Guinea-pig 98.—Put on oats, hay, and water. Died on 29th day. Necropsy: Hemorrhages around wrists, knees, into muscles of lower jaw, and into gums at base of lower molar teeth. Fractures of lower epiphysis of radius and ulna of left forefoot. Extensive swelling of wrists and knees. Looseness of all

the lower molars and of the last two upper molars of both sides. Enlargement of costochondral junctions with hemorrhage between ribs. The abdominal organs show postmortem changes, the animal having been dead for 2 days before necropsy.

As far as macroscopical examination could determine, these lesions were identical with those produced by milk but more extensive.

Three guinea-pigs were fed on bread, hay, and water. They averaged 140 gm. in weight. Lesions of scurvy appeared on the 25th day. Two died on the 34th and 35th days, respectively; the third, placed on a vegetable diet on the 32nd day, recovered. The lesions were midway in intensity between those of the milk- and those of the oat-fed guinea-pigs.

Three guinea-pigs weighing 130 gm. each were fed on bran, hay, and water. They died about the 31st day, all having looseness of the molar teeth, hemorrhages into the gums and jaw muscles, besides extensive hemorrhages in the fore- and hind-leg muscles. The lesions were more extensive in character than those found with any other diet.

GUINEA-PIGS ON MILK AND OATS

In a preceding paragraph it was noted that Funk prevented scurvy in guinea-pigs by adding 50 c.c. of raw milk daily to an oat diet. We fed 3 guinea-pigs, weighing 140, 155, and 170 gm. respectively, with all the pasteurized milk they could consume plus oats. The first lesions were observed on the 19th, 25th, and 26th days, in somewhat the same order as their weights. The symptoms were as severe as those produced on an oats diet alone. However, the animals gained greatly in weight compared with those fed on oats—that is, from 80 gm. on milk to 200 gm. on oats—before the onset of symptoms, and it may be that this factor prevented their early death, all of them living over 2 months on milk and oats diet. We have here added to a food (milk) which produces a mild degree of scurvy, a food (oats) which causes severe symptoms with death and have as a result a condition somewhat midway between those produced by each alone. This may be due to the weight increase, altho it does not follow that substances which maintain body weight are necessarily identical with antiscorbutic substances. Further experiments are being carried out which may offer some conclusion.

GUINEA-PIGS FED ON GOATS' MILK

Because of the ease with which a sufficient amount can be obtained, cow's milk has been oftenest used in milk-diet experiments. Whether the condition produced by feeding cow's milk would be representative of those produced by diets of milk from other animals is a question which led us to test goat's milk. Six guinea-pigs weighing from 110 to 145 gm. each were fed on fresh goat's milk, one set for 80 days, a

second for 44 days. The animals developed normally with no clinical symptoms of scurvy. No microscopical study of these animals was made.

TEMPERATURE AND LEUKOCYTE COUNT

The temperature of 9 guinea-pigs fed on pasteurized milk or milk with streptococcus broth (3 were in the scorbutic outbreak in the laboratory and 3 were controls) was taken during the experiments. The average temperature of the first series was 102.6 F., of the second 102.8 F., and of the controls 102.8 F. In 2 animals fed on milk and streptococcus broth the temperature was 104.4 F. and 104.6 F. on the first day that lesions were palpable. In other animals the temperatures averaged about 103 F. on the first day of symptoms. With the two exceptions, the highest figure was 103.8 F. It is apparent that experimental scurvy is a nonfebrile disease in the majority of affected animals.

Gulland and Goodall²² found the leukocytes of guinea-pigs to be 9,170 to the cubic centimeter and they quote Burnett as finding the number to be 9,000 to the cubic centimeter. Our observations on 5 guinea-pigs with scurvy gave us the average leukocytic count as 8,000, with high and low counts of 9,800 and 7,200. The number of leukocytes did not increase much with the appearance of symptoms, the average before lesions developed being 7,500 and afterwards, 8,200. In a sixth guinea-pig the count was 8,500 white cells to the cubic centimeter before lesions appeared, 10,000 the day swollen joints were palpated, and 12,800 three days later when the animal was killed. The average white-cell count of 3 control guinea-pigs was 8,200. Experimental scurvy, therefore, produces no appreciable leukocytosis in the majority of affected animals. The following protocol is typical of the temperature and of the leukocytic count:

Guinea-pig 16.—Commenced feeding on milk and streptococcus broth, July 23. Throat swabbed with streptococcus broth daily. July 27, T. 102.5; July 29, T. 102.6; July 30, T. 103; July 31, T. 102.4, white-cell count 8,000; August 1, T. 101.6; August 4, T. 103; August 5, T. 102.1, white-cell count 8,600, both wrist joints swollen; August 6, T. 103, white-cell count 8,500, right wrist greatly swollen; August 8, T. 103.4, white-cell count 9,500; August 11, T. 102.2, white cells 8,200; August 13, T. 102.6, right knee swollen in addition to wrists; August 15, T. 102.6, white cells 6,400; August 18, T. 103, white cells 5,500, both knees and wrists swollen. Placed on vegetable diet. September 12, chloroformed. Necropsy: Wrist joints swollen and ankylosed. Knee joints swollen and firm with old hemorrhages in periarticular tissues. All lymph glands swollen. Lungs contained areas of congestion. Other organs normal.

²² The Blood, 1912.

MICROSCOPICAL PATHOLOGY

In an earlier study by Le Count and Jackson²³ of the histologic changes in the bones and joints in a disease of guinea-pigs resembling scurvy and caused by feeding milk, chiefly late changes were considered. As a careful consideration of the primary alterations in this condition has generally been neglected, it seemed that a systematic study of them in conjunction with that of the later changes might be of value. In the present work some of the animals previously studied are included.

Tissues from 25 guinea-pigs were examined microscopically and those from 19 were selected with reference to securing a series as regards the periods of time elapsing between the beginning of feeding and the death of the animal, the shortest period being 9, the longest 69, days. The duration of the disease in the remaining 6 is not known exactly. Six of the 19 guinea-pigs were killed within the first 3 weeks after feeding was begun and before there were any clinical manifestations of the disease; 3 guinea-pigs were killed 21 days after the beginning of the experiment, or at about the time that symptoms first appeared in the milk-fed animals. All the animals except 3 from which tissues were examined microscopically, were fed on milk and hay without green vegetables, 2 were fed on oats, hay, and water, and 1 on casein, hay, and water.

The parts selected for examination were knee joints from 15 guinea-pigs, ribs from 11, the lower jaw from 6, wrist joints from 4, and the elbow joint from 1. Sections of the skin were also examined in a few instances. The material was fixed in Zenker's fluid and imbedded in paraffin. Serial sections were made from portions of some of the joints. Sections were stained by the usual methods, hematoxylin and eosin, phosphotungstic acid hematoxylin, and by the Wright and Giemsa methods. Wright's stain was much used because it gave excellent results with the bone marrow and also because it requires less manipulation of the sections, a distinct advantage in staining sections containing bone, which is easily detached from the slide.

Changes were found in sections from all but 2 of the animals examined. Hemorrhage was one of the earliest as well as most constant alterations observed. More or less necrosis and small infarct-like lesions were also present in tissues from some of the animals fed for a short period of time. Protocols and descriptions of the histologic changes found in 3 such animals follow:

Guinea-pig 182.—Commenced feeding on pasteurized milk January 28. Weight 140 gm. February 6, weight 210 gm. Killed. Hemorrhage into the muscles of the left thigh and below the left knee, otherwise no gross changes.

In some sections of the knee joint there was a necrotic region from 2 to 3 mm. in its longest dimension, which was parallel with the epiphyseal cartilage in the lower end of the diaphysis of the femur. In all the sections of this joint examined there was more or less change in the bone marrow and bone trabeculae adjacent to the epiphyseal cartilage, the marrow being more affected.

²³ Tr. Chicago Path. Soc., 1914, 9, p. 189.

Small hemorrhages; fragmentation of nuclei, and lessened staining properties. Cartilage unchanged. In some sections there was a much distended vein, or sinus, 5 mm. above the epiphysis, and at about the middle point of the shaft there appeared to be a thrombosis of a large vein. Numerous small hemorrhages in the periosteum covering the posterior surface of the femur and a rather large hemorrhage about the large vessels posteriorly which had extended for a considerable distance along some of the intramuscular septa. The place in the vessel wall from which this hemorrhage took place was not found. A less marked but similar condition was present in the upper end of the tibia.

Guinea-pig 163.—January 28, began feeding on milk and hay without green vegetables. Weight 145 gm. February 7, weight 175 gm. Killed. Small hemorrhage over the patella of the left knee, otherwise no gross changes. A large number of sections from one knee joint examined. Numerous small hemorrhages and one of considerable size in the tissues outside of the joint posteriorly, all of these being in the immediate neighborhood of veins or capillaries. The largest hemorrhage was about a vein of rather large size, in the wall of which were definite changes. Marked thinning of the wall (Fig. 3), as tho the wall as a whole had partially melted away leaving few traces. In this portion of the wall were many small round bodies resembling cocci (Fig. 3), which stained a deep blue by the Wright and Giemsa methods. These bodies were also present in the lumen of the vessel and in the inner layers of the more normal portions of the wall. In the lumen adjacent to the thinned portion of the vessel wall was a small amount of a finely granular pink-stained material. At one point in the lining of the joint cavity just beyond the cartilage was a minute region of surface necrosis (Fig. 4) from which a small triangular mass of eosin-stained material resembling fibrin extended into the cavity. In the midst of this mass were a number of the coccus-like bodies. No inflammatory reaction in or about these regions of hemorrhage and necrosis, with the exception of many eosinophile cells, both mononuclear and polymorphonuclear, which were particularly numerous around blood vessels and in the bone marrow. Changes in some of the muscle fibers, particularly those attached to the periosteum. Portions of the fibers (Fig. 5) partially or completely disorganized and replaced by a small amount of finely granular material.

Guinea-pig 154.—September 3, began feeding on milk and hay without green food. Weight 120 gm. September 17, weight 150 gm. Killed. No gross changes present in any of the organs. Sections of both knee joints, the lower jaw, and two ribs were examined. A well-defined region resembling an infarct (Fig. 6) in about the middle portion of the shaft of the tibia and just anterior to the greatly dilated central vein draining the upper part of the bone. A series of sections through only a part of the lesion was obtained and in these no occluded vessel was found. There were hemorrhages and much fibrin present in this region but no necrosis. The vein in the neighborhood of this lesion occupied two-thirds of the diameter of the shaft at this level. Hemorrhages and a small region of necrosis occurred in the bone marrow and bone at the lower ends of the diaphysis of the femur near the periphery posteriorly. In the other knee joint there were also small hemorrhages in the bone marrow close to the epiphysis and about the veins in the popliteal space. An infarct-like lesion similar to that in the tibia was present in the pulp of one of the incisor teeth. In sections of one of the ribs there was hemorrhage into the marrow near the costochondral junction with a small amount of necrosis at the periphery.

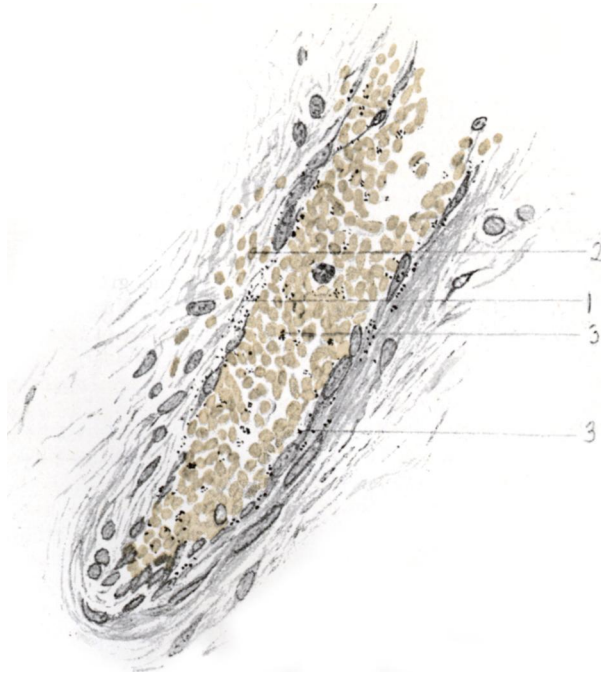


Fig. 3. A portion of the wall of a vein in the popliteal space of Guinea-pig 183. The figures indicate (1) a thinned portion of the wall, (2) red blood corpuscles outside the vessel wall, and (3) bacteria.

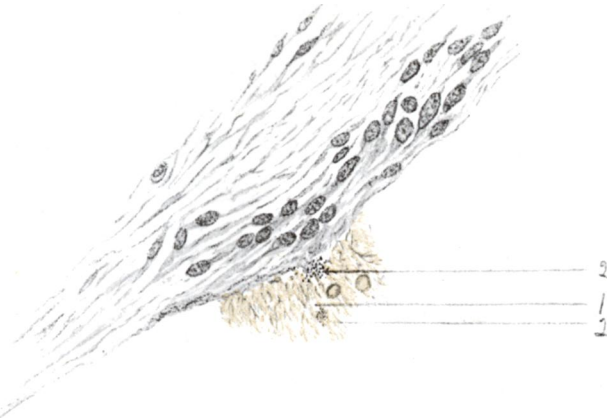


Fig. 4. A portion of the lining of the joint cavity of Guinea-pig 183. The figures indicate (1) a small mass of fibrin-like material, and (2) a clump of bacteria.



Fig. 5. A portion of the muscle and periosteum from a section of the knee joint of Guinea-pig 154. The figures indicate (1) the periosteum, (2) normal muscle fibers, (3) degenerated muscle fiber, and (4) a capillary.

In charting the lesions observed in the tissues from all these animals it was discovered that hemorrhage was present in all the guinea-pigs in which there were any alterations. They were widely distributed, being found in the muscles, bone marrow, periosteum, tooth pulp, and occasionally in the skin.

Hemorrhages occurred in the bone marrow at many points, but they were especially frequent at the end of the diaphyses of long bones, near the costo-

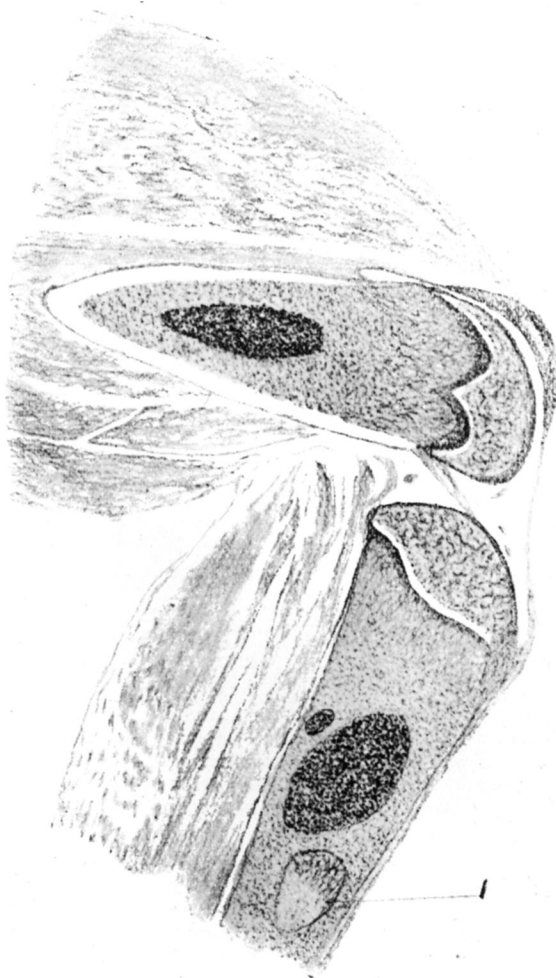


Fig. 6. A section through the knee joint of Guinea-pig 154. The figure (1) indicates a small infarct-like lesion in the bone marrow.

chondral junctions of the ribs, about blood vessels near the point where they pass through the compact bone of the shaft of long bones, and in the bone marrow of the lower jaw near the teeth, and also in the tooth pulp. Outside the bone they were present in the muscles of the leg and thigh, but particularly about the large vessels in the popliteal space, in the muscles over the ribs close to the costochondral junctions, and in the muscles of the lower jaw, particularly about the attachment of the masseter muscle to the posterior portion of the inferior maxilla. When the disease was of long standing, there were deposits of pigment, some of it contained in large endothelial cells, with the surrounding tissues more or less changed, marking the site of the old hemorrhage.

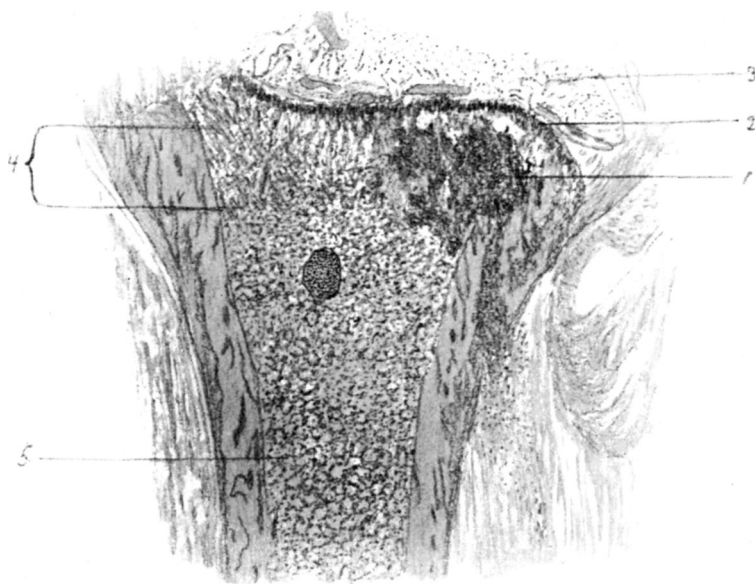


Fig. 7. A section through the lower end of the femur of Guinea-pig 7. The figures indicate (1) a region of necrosis, (2) epiphyseal cartilage, (3) the marrow of the epiphysis, (4) a region of osteoid tissue, and (5) the marrow of the shaft.

Hemorrhages varied as to size and character. There was often a uniform scattering of red corpuscles through rather large regions of bone marrow, this being usually associated with a decrease in the lymphoid elements. Hemorrhage of this kind was frequently found in the marrow of the wrist bones and in the tooth pulp. Small and well-circumscribed hemorrhages occurred early in the disease and in the milder forms. These as well as some of the larger hemorrhages when examined early in the disease were found associated with definite alterations in the walls of veins. In general the large hemorrhages occurred in the severe and later forms of scurvy and appeared to take place gradually. In some specimens examined the popliteal space was filled with blood and the muscles and spaces between thoroughly infiltrated with blood. Infarction of the upper end of the tibia or of the lower end of the femur was accompanied by large hemorrhages at the epiphyseal junctions, and usually also at about the middle point of the shaft (Figs. 7 and 8) both in the marrow and in the tissues outside the bone where the vessels pierce the compacta. With marked changes in the teeth there was often great dilatation of the veins of the pulp attended by more or less hemorrhage into the pulp (Fig. 9) and also into portions of the alveolar process immediately in contact with the teeth. Holst and Fröhlich observed petechiae in the skin, which with one exception were found in the follicles of the vibrissae. Altho looked for with considerable care in animals having severe lesions elsewhere, no hemorrhages in this location were found. In a few guinea-pigs, however, especially those subjected to experiment for the second time, we observed bleeding into the inner layers of the skin. These lesions varied in size from that of pinhead size to those 1.5 cm. in diameter. Microscopically the hemorrhage was just external to the muscular layer. In

these regions in Guinea-pig 299 there were numerous large endothelioid cells, many of them multinucleated, but no polymorphonuclear leukocytes.

The axillary, inguinal, and popliteal lymph glands were enlarged and often hemorrhagic. In sections it appeared that the hemorrhage was entirely into the lymph tissues, and in some cases practically all of the red blood corpuscles had been taken up by, or were attached to, large lymphocytes or endothelial cells. In more advanced stages of the disease these cells contained pigment. In the adipose tissue about the popliteal lymph gland from one of the animals a considerable number of multinucleated giant cells were found.

Lesions having the shape, location, and characteristics of infarcts were found in the ends of the diaphyses of long bones. In one of the animals fed on oats, hay, and water (166) there was complete infarction of the upper end of the tibia. As previously mentioned, we also observed small well-defined ovoid lesions in the bone marrow (Fig. 6) and in the pulp of the teeth which resembled infarcts. There was some hemorrhage with fibrin and edema of the tissue, but no occluded vessels were found. In a few instances clots with fibrin-formation, disintegration of nuclei, and beginning organization were present in the large central veins of the long bones; also rather frequently in this location and in the tooth pulp (Fig. 9) greatly distended veins were seen, about which there was more or less hemorrhage.

Guinea-pig 165 illustrates both the severity of the changes that developed in guinea-pigs fed on oats and water and their similarity to those produced by feeding milk.

Guinea-pig 165.—August 22, began feeding on oats, hay, and water without green vegetables. Weight 130 gm.

Sept. 9.—Weight 170 gm. Congestion of the gums at the base of the incisor teeth.

Sept. 14.—Wrists swollen and painful, also the knees. Gums congested as on the 9th. Weight 180 gm. From this time on there were a gradual loss in weight, and increased swelling and painfulness of the joints and muscles until on the 22nd the animal was unable to walk.

Sept. 29.—Died. Necropsy: Lungs slightly congested. Spleen about twice normal size. Lymph glands large and congested. Hemorrhages into the muscles of the thigh. Both wrists markedly swollen and red. The heads of both tibiae enlarged, with surrounding hemorrhages. Costochondral junctions greatly enlarged and the last molar teeth slightly loosened.

In sections of the knee joint, huge hemorrhages could be seen with the unaided eye in the tissues about the large vessels posteriorly and in and between the muscles of the leg and thigh, also lightly stained regions, one of which comprised a zone 2 mm. wide adjacent to the epiphysis in the bone marrow of the lower portion of the diaphysis of the femur. Most conspicuous were the hemorrhages present in all parts of the sections but particularly in the popliteal

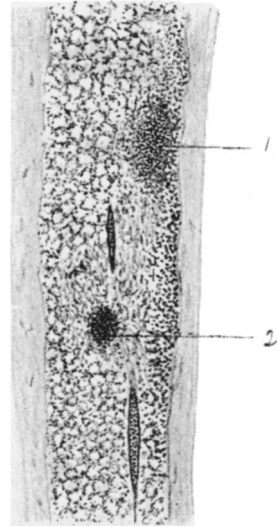


Fig. 8. A section through the shaft of the femur of Guinea-pig 7. The figures indicate (1) a region of hemorrhage surrounded by osteoid tissue and (2) a region of hemorrhage surrounded by marrow.



Fig. 9. A section through a molar tooth of Guinea-pig 165. The figures indicate (1) an alveolar process, (2) dentin, (3) pulp, and (4) a hemorrhage.

space, in the vicinity of the lower epiphysis of the femur, and in the periosteum. The largest hemorrhages were outside the bone.

There was a decided decrease in the lymphoid cells in the lightly stained portions of the bone marrow (Gerustmark) and in their place were many spindle-shaped cells, among which were scattered red blood corpuscles. A golden-brown pigment was present in some of these cells, especially near the epiphyseal cartilage, where the other changes just mentioned were most pronounced. At either margin of the bone and between the zone of osteoid tissue and the epiphyseal cartilage there were narrow strips of necrosis extending inward along the cartilage about 1 mm. Opposite these regions of necrosis were small regions of rarefaction of the marrow in the epiphysis similar to that found in the shaft. In places there was an absence of the compacta for a distance of a few millimeters above the epiphyseal cartilage. The periosteum covering the lower portion of the femur was greatly thickened and contained many small hemorrhages.

Marked changes occurred in the tissues immediately about the joint, particularly in the regions of large hemorrhages posteriorly. There was complete disappearance of muscle fibers from areas of considerable size in the immediate vicinity of large hemorrhages, and in other places of a few or single fibers, adjoining fibers being little or not at all changed. These were replaced by spindle-shaped connective-tissue cells, some containing a golden-brown pigment, and in some places by a granular eosin-staining material. In the less altered portions of the muscles there was a great increase in the number of nuclei. Sometimes 3, 4, or 5 small deeply stained nuclei lay close together in a row. In places large irregular shaped masses of chromatin were found in the muscle fibers.

In sections of the lower jaw hemorrhages were numerous in the bone marrow, periosteum, and tooth pulp. The hemorrhages in the periosteum were especially noticeable in the portions covering the alveolar process. There was great dilatation of some of the vessels in the pulp of the molar teeth at about their middle with considerable hemorrhage about them (Fig. 9).

Descriptions of the alterations found in tissues from Guinea-pigs 16 and 3, the protocols for which have been given, furnish very good examples of the appearances obtained in the later stages of the process when healing is in progress, or, as in some parts, complete.

In Guinea-pig 16 the line of junction between the bone and costal cartilage instead of being well-defined was irregular (Fig. 10) because of small trabeculae of cartilage which projected towards the marrow of the bone for a short distance. There were many of these as wide as 2 or 3 cartilage cells. They extended out to about the same distance from the end of the cartilage and for almost the entire width of the shaft. A thin layer of necrotic tissue covered them; in other words, there had been a necrosis of the costal cartilage. Farther towards the shaft of the bone was a narrow zone of loosely aggregated fibroblasts—osteoid tissue—containing irregular masses of cartilage and bone. This was two or three times the width of the trabeculae of cartilage previously mentioned. The necrosis was always most marked at the edges and least marked at the middle, therefore the sections cut from the surface possessed the largest amounts of necrosis. In the periosteum and surrounding muscles were hemorrhages of considerable size. The changes in sections of the knee joint were similar to those described in the rib. In the wrist joint, however, the distal end

of the diaphysis of the radius was occupied by a very mature callus—heavy spongy bone—with large amounts of pigment in the marrow.

In Guinea-pig 3 in sections of a rib on either side masses of cartilage close to the periosteum had replaced the break caused presumably by necrosis, and spongy bone formed the middle of the callus. The bone marrow in this region was fibrous with almost no lymphocytes present. The tissue surrounding this portion of the rib contained old hemorrhages and many cells contained a golden-brown pigment. Healing had reached a somewhat advanced stage in the muscles and other tissues surrounding the knee joint. The regions of hemorrhage, especially about the large vessels posteriorly, were quite thoroughly grown through with large irregular stellate and spindle-shaped cells with large nuclei containing one or more nucleoli. Some of these cells stained very faintly. Small round cells were also present but were less numerous. There was more or less infiltration in these cells about the blood vessels (Fig. 11) even in the more normal parts of the section. The large cells also filled in small defects in the muscles at various points. They frequently contained pigment. A noticeable feature was the swollen endothelium of some of the blood vessels with, seemingly at least, an increase in the number of cells. The endothelium in places in the larger vessels had a vacuolated appearance.

In the earliest lesions there was no change in the tissues at the site of hemorrhage. Necrosis was not a prominent feature until after the third week in milk-fed guinea-pigs. However, in the earliest specimens studied there were some fragmentation of nuclei, loss of staining properties of the cells, and formation of a fine fibrin network in places in the bone marrow of the ends of the diaphyses of some of the long bones with occasional small hemorrhages in some, accompanied by hemorrhages of considerable size in others. Definite small regions of necrosis were also occasionally present in this location. Changes were noted in some of the veins outside the bone in the 9-, 10-, and 14-day guinea-pigs. The walls of veins in the immediate vicinity of hemorrhages were sometimes thinned in certain places with separation of the parts by red blood corpuscles and occasionally a complete break. The appearance was that of a complete disappearance or melting away of parts leaving scarcely a trace. There was a small amount of nuclear fragmentation, and large numbers of coccus-like bodies were present in the wall and in the lumen.

From a histological study of this disease it appears that the changes were due to some agent of a mildly destructive nature apparently acting chiefly on the circulatory apparatus to cause the hemorrhages so frequently encountered in this condition, but likewise causing alterations in the muscle fibers, bone, and bone marrow. In the latter stages especially it was impossible to decide how much destruction was due to the primary cause and how much was secondary to hemorrhage, but a study of the early stages makes it evident that at least a part of the

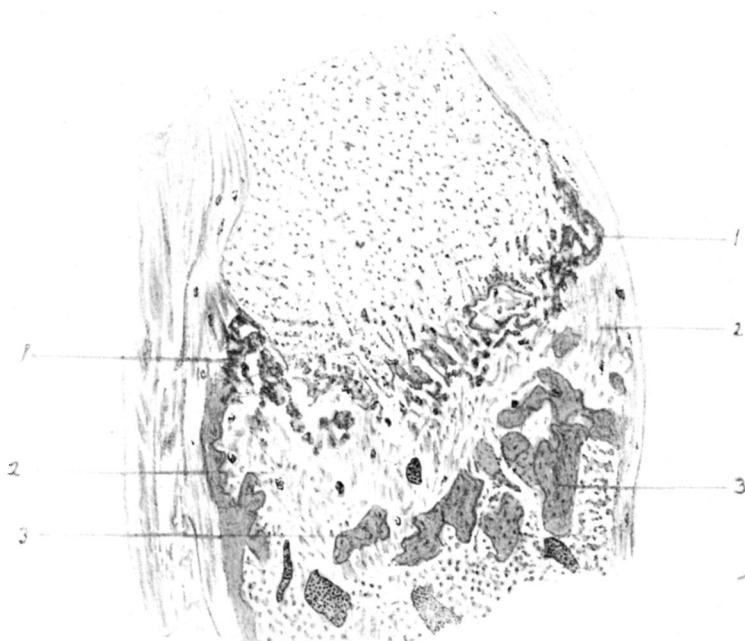


Fig. 10. A section through the rib of Guinea-pig 16. The figures indicate (1) a zone of necrosis near the cartilage, (2) a zone of osteoid tissue, and (3) irregular trabeculae of bone.



Fig. 11. Infiltration about a small blood vessel in the muscles of the leg in Guinea-pig 3.

changes in the muscles and bones were not the result of hemorrhage. Certain features of the process seem to indicate that we may have been dealing with a mild infection.²³ It is true that a marked inflammatory reaction was lacking, but it is a localized process, there being certain favorite points of attack, some and not all points being affected at the same time, except when the disease is severe and prolonged. Altho the most prominent feature was the bone changes, the disturbance did not seem to be with the mechanism of bone-formation as a whole, but disturbances at certain definite points interfered with normal



Fig. 12. A section through the middle of a rib of Guinea-pig 11. The figures indicate (1) a small region of necrosis, (2) blood vessels, (3) bone trabeculae, and (4) cartilage.

processes there. For example, marked alterations might occur in one bone of the knee without the others' being similarly affected or some and not all the ribs might undergo changes. Moreover, changes of the same character occurred outside the bones in the muscles and other soft tissues. Naturally the attention was not so forcibly attracted to alterations in these tissues as the results were not so momentous for the growing organism. The frequent occurrence in these lesions of bodies having the staining properties and other characteristics of bacteria also strengthens the impression. It likewise seems

²³ Jackson and Moody: Jour. Infect. Dis., 1916, 19, p. 526.

probable that the hemorrhages, so conspicuous in this condition, might have been due to localized injuries to the vessel walls and that the agent causing the disturbance in the blood vessels might also have been responsible at least in part for the changes in the ends of the diaphyses of long bones, in the muscles, and elsewhere, aside from rather large numbers of eosinophile cells in these regions where there was no inflammatory reaction in the tissues.

Necrosis occurred at the costochondral junctions of the ribs and at the ends of the diaphyses of long bones affecting chiefly the bone and bone marrow, to a less extent the cartilage, and was most marked at the periphery, where the compact bone was sometimes completely destroyed for a short distance. As might be expected fractures at these points were rather frequent. The margins of the cartilages were uneven, the arrangement of the cartilage cells irregular, and, especially at the costochondral junctions, the cartilage bulged towards the marrow cavity. In the guinea-pigs fed on oats and hay there was almost complete necrosis of the pulp of the incisor teeth, also more or less necrosis in the pulp of the molars.

The appearances observed in the process of healing naturally depended largely on the extent of the earlier changes and the duration of the disease. If the alterations had been slight there might simply be changes in the character of the bone marrow near the costochondral and epiphyseal junctions with slight increase in width at these points and evidences of old hemorrhages both within and without the bones. Following the marked early changes, large hemorrhages, necroses, and fractures, there was more or less deformity, with enlargement of wrist and knee joints especially, and an increase in width of the costochondral junctions. The reparative process in bone in this disease was such as would normally take place after an injury of like severity and of slight inflammatory character, by the formation of osteoid, cartilagenous, and bony callus. One observed in the region of the epiphyseal cartilage a fibroid marrow, marked irregularity of the diaphyseal margin of the epiphyseal cartilage (Fig. 13), and at the periphery osteoid, cartilagenous, or bony callus (Figs. 12, 13, and 14) according to the stage in the process.

Changes in the muscle fibers are of interest. These alterations were noticed particularly in the muscles of the leg and thigh. Early in the disease and also in the later moderately severe cases a very small or considerable portion of the fiber might be completely disintegrated while other parts and adjacent fibers appeared unchanged.



Fig. 13. A section through the upper end of the tibia of Guinea-pig 1. The figures indicate (1) bone marrow of the epiphysis, (2) trabeculae of bone, (3) epiphyseal cartilage, and (4) osteoid tissue.

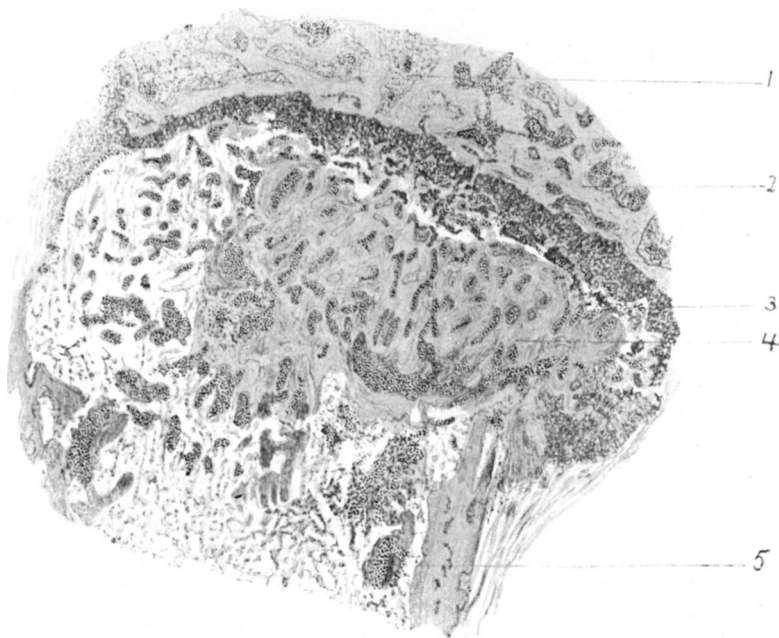


Fig. 14. A section through the upper end of the tibia of Guinea-pig 11. The figures indicate (1) marrow of the epiphysis, (2) trabeculae of bone, (3) epiphyseal cartilage, (4) callus made up of dense spongy bone, and (5) marrow of the shaft.

The degenerative process here was of the same character as that in the blood vessels and appeared very frequently to be entirely independent of hemorrhage. Lesions in the muscles appeared as early in the disease as hemorrhages and their severity varied with that of the lesions elsewhere.

SUMMARY

Experimental scurvy was produced in guinea-pigs by diets of pasteurized, raw, boiled, skimmed, and condensed milk, streptococcus broth and milk, milk and green vegetables, thyroid extract and milk, casein and water, oats, bread, and bran. The addition of calcium lactate to milk or the injection of calcium lactate into guinea-pigs on a milk diet did not prevent scurvy. A cream diet, and a diet of olive oil added to milk, produced a "fat constipation" with early death. Daily injections of olive oil into animals on a milk diet had no antiscorbutic effect.

Mixed broth cultures of *Streptococcus viridans* and *Streptococcus hemolyticus*, water, lactose water, and lime water did not produce scurvy.

In a series of 6 guinea-pigs fed on goat's milk for over 40 days, no symptoms of scurvy developed.

In guinea-pigs fed on milk, the clinical symptoms, in brief, were preliminary loss of weight, swelling of the wrist and knee joints, occasionally of the costochondral junctions, ankle, and elbow joints, and occasionally hyperemia of the gums with dullness of the lower incisors. Fractures of the long bones near the epiphyseal ends were common; fragility of the bones was more or less marked. Exostoses and deformities were frequent, especially when the milk diet was continued for several weeks.

The average time for the onset of symptoms with pasteurized milk was 19 days. With other milk diets this varied from 11 to 19 days. The earliest lesion was observed on the 10th, the latest on the 29th day.

The disease was afebrile and produced no great increase in leukocytes.

The chief pathologic lesions noted post mortem were hemorrhages, which were found in the muscles, bone marrow, more frequently at the ends of diaphyses, tooth pulp, costochondral junctions, and occasionally in the skin and lymph glands; enlargements of the ends of the long bones, especially the lower ends of the radius and ulna, the upper end of the tibia, and the costochondral junctions; and swollen lymph

glands, especially the inguinal and axillary. The enlargement of the bones was often accompanied by fractures near the epiphyseal junctions.

Microscopically the earliest lesions observed were slight amounts of necrosis and hemorrhage in which coccus-like bodies were frequently demonstrated, the only evidences of inflammation being slight fibrin-formation and the presence in the surrounding tissues of large numbers of mononuclear and polymorphonuclear eosinophile cells. Small infarct-like lesions were also found early in the process. The later changes were those customarily found and previously described in experimental scurvy in guinea-pigs.

Cultures of the heart blood from guinea-pigs with scurvy from milk diet were sterile, and passage of blood from these animals to normal animals did not produce the disease.